

BEST AVAILABLE COPY

Linoleic-acid-enriched diet: long-term effects on serum lipoprotein and apolipoprotein concentrations and insulin sensitivity in noninsulin-dependent diabetic patients^{1,2}

Robert J Heine, Cees Mulder, Corrie Popp-Snijders, Jan van der Meer, and Ed A van der Veen

ABSTRACT Long-term (30 wk) effects on serum lipoproteins and insulin sensitivity of two diets, one with a low polyunsaturated to saturated fat ratio (P:S 0.3) and one with a P:S of 1.0, were compared in 14 patients with noninsulin-dependent diabetes mellitus (NIDDM) in a crossover study. Total and LDL-cholesterol levels declined by 7.6% ($p < 0.01$) and 9.8% ($p < 0.01$), respectively, during the high P:S diet. VLDL-, HDL2-, and HDL3-cholesterol; triacylglycerol; and apolipoprotein A1, A2, and B levels were not affected by the change in P:S. Despite a modest increase of insulin-mediated glucose disposal at physiologic insulinemia during the high P:S diet, no influence was seen on glycemic control, and on blood glucose, plasma insulin, and C peptide responses to mixed meals.

In conclusion, a linoleic-enriched diet in patients with NIDDM causes a less atherogenic lipoprotein profile but does not influence glycemic control and carbohydrate tolerance. *Am J Clin Nutr* 1989;49:448-56.

KEY WORDS Linoleic acid, polyunsaturated fatty acids, diet, insulin sensitivity, lipids, cholesterol, LDL-cholesterol, diabetes, apolipoproteins, lipoproteins

Introduction

Atherosclerosis is a major cause of death and morbidity in diabetic patients (1-3). Because of this predisposition, attention has been focused on lipoprotein and apolipoprotein metabolism in diabetics. Hyperlipidemia is frequently encountered in noninsulin-dependent diabetics and is considered as a major determinant of atherosclerotic complications (4-6). Besides the known hypertriacylglycerolemia, alterations in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) metabolism have attracted more attention as they may be more intrinsically related to the pathogenesis of atherosclerosis (7, 8). Also obesity, insulin resistance, and hypertension have been shown to have a predictive value for the development of coronary heart disease (1, 9, 10). These risk factors can be influenced by dietary treatment. The World Health Organization (WHO) expert committee on diabetes mellitus (11) recommends in their latest report a dietary fat restriction to approximately 30 energy percent. Moreover, they advised diabetics to substitute foods containing polyunsaturated vegetable oils for saturated fats. Consequently the carbohydrate content has to be raised to 50 energy %, enriched with natural dietary fibers. This prudent diet has also been recommended to the general population to reduce the risk of atherosclerotic events (12).

Polyunsaturated fatty acids have been claimed to enhance the sensitivity to insulin in diabetic patients. Kinsell (13) observed hypoglycemic episodes when linoleic acid was substituted for saturated fatty acids in the diets. A study in noninsulin-dependent diabetics demonstrated improvement of the glucose tolerance in women using a diet with a high ratio of polyunsaturated to saturated fatty acid (P:S) (14).

Despite these studies it is still very hard to draw firm conclusions as to the effect of polyunsaturated fatty acids on lipid levels and insulin action in general, and of linoleic acid in particular, because studies were either of short duration (13) or were comparing diets that differed in their relative proportions of carbohydrate and fat (14).

We decided therefore to study the long-term effects of a diet enriched with linoleic acid to a P:S of ~1, without further altering the composition of the diet, on serum lipoprotein and apolipoprotein levels and insulin sensitivity in noninsulin-dependent diabetic patients.

¹ From the Departments of Internal Medicine, Clinical Chemistry, and Endocrinology, Free University Hospital, Amsterdam, The Netherlands.

² Address reprint requests to RJ Heine, Department of Internal Medicine, Free University Hospital, De Boelelaan 1117, Amsterdam, The Netherlands.

Received November 12, 1987.

Accepted for publication March 22, 1988.

BEST AVAILABLE COPY

DIETARY LINOLEIC ACID IN NIDDM

449

TABLE I
Clinical and biochemical details at time of recruitment of the patients who completed the study

Subject	Sex	Age	BMI*	Known diabetes duration	Therapy	Fasting HbA1 blood glucose†	Fasting plasma insulin	Fasting plasma C peptide	Total cholesterol	LDL cholesterol	HDL2 cholesterol	Triacylglycerol
		y	kg/m ²	y		mmol/L (%)	mU/L	nmol/L	mmol/L	mmol/L	mmol/L	mmol/L
1	M	46	29.3	8	diet	10.8(10.4)	18	0.54	5.06	3.29	0.19	2.5
2	F	40	24.9	16	diet	13.0(11.6)	9	0.27	8.31	6.62	0.26	1.7
3	F	55	34.0	2	diet	6.6(6.4)	18	0.73	7.44	5.66	0.25	2.1
4	M	30	25.3	3	diet	9.6(8.8)	11	0.30	3.55	2.18	0.23	1.2
5	F	43	24.7	5	diet	12.2(10.5)	24	0.38	6.67	4.99	0.32	3.1
6	F	59	25.6	2	diet	8.1(6.4)	15	0.75	5.97	3.67	0.24	5.4
7	F	61	22.8	3	glibenclamide	9.4(9.9)	12	0.47	4.21	2.53	0.35	1.7
8	F	46	19.8	9	tolbutamide	11.5(10.0)	15	0.28	4.33	2.98	0.40	1.6
9	M	70	28.4	8	gliclazide	7.7(7.6)	16	0.57	5.45	4.45	0.16	1.5
10	M	69	19.5	9	glibenclamide	7.5(10.3)	14	0.29	3.83	2.01	1.13	0.5
11	M	64	26.4	5	tolbutamide	11.5(8.6)	10	0.43	4.16	3.03	0.23	1.0
12	M	47	24.6	8	glibenclamide	6.0(6.0)	11	0.30	3.94	2.53	0.29	0.8
13	M	47	25.6	9	gliclazide	10.2(6.6)	21	0.87	5.60	3.96	0.21	3.1
14	M	49	24.9	6	glibenclamide	9.5(8.4)	10	1.12	8.14	5.96	0.35	3.6

* Body Mass Index = body wt/m².

† Percent is given in parentheses.

Subjects and methods

Subjects

Seventeen patients, 8 women and 9 men, were recruited. Inclusion criteria for this study were age < 70 y and treatment consisting of diet only or a stable dose of sulfonylurea for at least 3 mo before the start of the study. Excluded were patients with other endocrine diseases, liver or renal disease as confirmed by biochemical findings, and those on treatment that would affect lipid or carbohydrate metabolism (eg, antihypertensives, corticosteroids, metformin, and diuretics). Patients were only admitted when it was to be expected that metabolic control did not require imminent alterations in treatment. All patients had a stable weight (< 5% different from that 3 mo before the start of the study) during at least 3 mo before the start of the study. Three patients dropped out during the first 3 mo after the start of the study, one man because of high alcohol intake that required special treatment and one woman because of breast carcinoma. In one woman metabolic control deteriorated, reflected by fasting blood glucose levels > 15 mmol/L, which required insulin treatment. Enrollment of the patients took place between April 1984 and July 1985.

All patients were diagnosed as being diabetic according to the criteria as recommended by the WHO study group on diabetes mellitus (11). The clinical characteristics of the patients who completed the study are given in Table 1. Before entering the study each patient gave informed consent and approval for the study was obtained from the Ethical Committee of the Free University Hospital was obtained.

Study protocol

The study consisted of two periods of 30 wk each in a cross-over design. During one period the P:S ratio in the diet was designed to be 0.3 and in the other period 1.0. The order of the dietary periods was randomized. At entry in the study the diets were individually planned to be isocaloric with the energy intake of the patient calculated from 1-wk dietary recalls. A com-

puter data bank of Dutch food composition was used to assess the diet compositions and to design the appropriate diet for the study. No attempt was made to adjust the habitual diet of the patient to the dietary guidelines, which were given at time of diagnosis of diabetes (ie, a diet consisting of 50–55 energy % carbohydrate, enriched with dietary fibers and 30 energy % fat with a P:S of 1). The composition of the diet and the cholesterol and fiber content were kept constant. Only the P:S of the diet was altered by substituting linoleic-acid-rich oils and fats for products rich in saturated fatty acids.

Diet compliance was controlled by 1-wk dietary recalls at 6, 12, and 24 wk during the two dietary periods. If necessary the diet prescriptions were adjusted. In addition, the fatty acid composition of the plasma cholestry-l-esters were determined at 6-wk intervals throughout the study and the lipid composition of the red blood cells at the end of the dietary periods.

The patients were seen at 6-wk intervals in the outpatient clinic after an overnight fast from 2200 the evening before, for the determination of body weight and blood pressure. Blood pressure was measured after a 15-min rest in a sitting position by one observer using the same mercury sphygmomanometer with a standard cuff. Diastolic blood pressure was recorded at Korotkoff phase V. The mean of three measurements was recorded.

Blood was withdrawn from an antecubital vein for the assessments of blood glucose, hemoglobin A₁ (HbA₁), serum lipids (total cholesterol, triacylglycerol, very-low density lipoprotein [VLDL] cholesterol, LDL cholesterol, HDL2 and HDL3 cholesterol), and apolipoproteins A1, A2, and B. After taking the blood samples, 100 IU heparin/kg body wt (Organon Teknica BV, Boxtel, Holland) was administered as an intravenous bolus. Twenty minutes later venous samples (EDTA as anticoagulant) were withdrawn for the assessments of post heparin lipolytic activity (PHLA), lipoprotein lipase (LPL), and hepatic lipase activity. Blood for measurement of ¹²⁵I-labeled insulin binding to red blood cells, according to Gambhir et al, was taken at 18 and 30 wk of each dietary period (15).

en-
its.
to-
the
con-
ien-
tu-

rm
ids
in-
of
red
4).
of
out
im-
tsi-

try,
th-

nal
am,

—
ion

TABLE 2
Nutrient composition of the reference (low P:S) diet and the linoleic-acid enriched (high P:S) diet (mean 24-h values of 1-wk dietary recalls)

	Low P:S diet				High P:S diet			
	Week 6	Week 12	Week 24	Mean	Week 6	Week 12	Week 24	Mean
Energy intake (kcal/d)	1666 ± 86	1746 ± 117	1826 ± 120	1746 ± 98	1641 ± 105	1752 ± 112	1738 ± 113	1710 ± 104
Protein								
animal (energy %)	10.7 ± 0.8	12.1 ± 0.8	11.6 ± 0.9	11.5 ± 0.7	12.8 ± 1.0	12.7 ± 0.9	12.1 ± 1.0	12.5 ± 0.9
vegetable (energy %)	4.8 ± 0.4	4.7 ± 0.3	4.7 ± 0.3	4.7 ± 0.3	5.0 ± 0.4	4.9 ± 0.4	4.8 ± 0.4	4.9 ± 0.2
Fat (energy %)	38.6 ± 2.1	38.9 ± 2.0	39.7 ± 2.3	39.1 ± 2.1	37.0 ± 1.7	38.3 ± 1.8	39.3 ± 1.5	38.2 ± 1.5
linoleic acid (energy %)	3.9 ± 0.4	4.1 ± 0.5	4.6 ± 0.4	4.2 ± 0.4	10.0 ± 0.8	10.9 ± 0.8	11.7 ± 0.8	10.9 ± 0.6†
P:S	0.31 ± 0.03	0.32 ± 0.03	0.35 ± 0.03	0.33 ± 0.02	0.83 ± 0.07	0.93 ± 0.08	0.97 ± 0.08	0.91 ± 0.06†
Cholesterol (mg/d)	261 ± 25	266 ± 28	290 ± 33	272 ± 25	217 ± 20	225 ± 21	223 ± 14	222 ± 13‡
Dietary fiber (g/d)	20 ± 2	21 ± 2	22 ± 2	21 ± 2	22 ± 2	21 ± 2	20 ± 2	21 ± 2
Carbohydrate (energy %)	40.2 ± 1.9	39.4 ± 1.9	38.8 ± 2.1	39.5 ± 1.9	39.9 ± 1.6	40.4 ± 1.6	39.4 ± 1.2	39.9 ± 1.3
Alcohol (energy %)	5.9 ± 1.4	5.1 ± 1.4	5.2 ± 1.4	5.4 ± 1.3	5.2 ± 1.3	4.0 ± 1.3	4.6 ± 1.2	4.6 ± 1.2

* $\bar{x} \pm 1$ SEM.

† $p < 0.001$ as compared with mean value during low P:S diet.

‡ $p < 0.05$.

Meal tolerance tests were performed in the fasting state at 0800 during the final 2 wk of the dietary periods. A standardized liquid mixed meal containing 56 energy % carbohydrate and 25 energy % protein (Clinifeed-protein rich®, Roussel, Hoevelaken, Holland) was taken in 5 min. Blood glucose and plasma insulin and C peptide were measured twice basally and at 15-min intervals for 3 h thereafter.

In vivo insulin sensitivity was assessed by constructing insulin dose-response curves in the fasting state, separated by at least 1 wk from the meal tolerance test, at the end of the dietary periods, by use of sequential glucose and insulin infusions at prefixed rates (16). Insulin was infused sequentially at 50, 150, and 500 mU · kg⁻¹ · h⁻¹ (Humulin R, Lilly, Indianapolis, IN) for 150, 120, and 120 min, respectively. Each insulin infusion was primed by an intravenous bolus injection of insulin of 0.1 × kg body wt × desired elevation of plasma insulin levels in mU. Glucose infusions at 6, 8, and 10 mg · kg⁻¹ · min⁻¹ were initiated 10 min later than the corresponding insulin infusions. Blood samples for blood glucose levels were taken each 5 min and for plasma insulin at 10-min intervals during the final 30 min of each glucose-insulin infusion. From these values the steady state plasma insulin levels were calculated. The metabolic clearance rate of glucose was obtained by dividing the glucose infusion rate by the mean blood glucose level during the final 30 min of each infusion.

Laboratory methods

Blood glucose was measured by a glucose oxidase method (Yellow Springs Instrument Co, Yellow Springs, OH). Plasma insulin was assessed by radioimmunoassay with human insulin as standard (Sorin Biomedica, Sallugia, Italy; sensitivity 2.5 mU/L; intraassay coefficient of variation 8.2%). Plasma C peptide was measured by radioimmunoassay with ethanol precipitation with human C peptide as standard (Novo Industri, Bagsvaerd, Denmark; sensitivity 0.06 nmol/L, intraassay coefficient of variation 8.1%) (17). Glycosylated hemoglobin was assayed by a microcolumn method (18). For the determination of triacylglycerol levels the glycerol phosphate oxydase-para amino phenozone (GPO-PAP) (human) was applied. Total cholesterol in serum and in the lipoprotein fractions was measured enzymatically using the Monotest kit (Boehringer Mann-

heim, GmbH, FRG). An IEC-B60 ultracentrifuge (Damon/IEC, Needham Heights, MA) was used for density gradient ultracentrifugation: VLDL was isolated at $d < 1019$ g/mL, LDL between $d = 1019$ and $d = 1063$ g/mL, HDL2 between $d = 1063$ and $d = 1.125$ g/mL, and HDL3 between $d = 1.125$ and $d = 1.210$ g/mL (19). The PHLA was measured according to a modified method of Lewis (20).

Post heparin plasma was incubated with Intralipid® (Kabi Vitrum, Stockholm, Sweden) and bovine serum albumin with a negligible free fatty acid content (Boseral, Organon, Oss, Holland) in a tris-HCl buffer (0.2 mol tris/L, 0.15 mol NaCl/L, pH = 8.5). For the determination of released fatty acids the enzymatic nonesterified fatty acid-C (NEFA-C) test (Wako, Osaka, Japan) was used (21). To determine hepatic triacylglycerol lipase (h-TGL), the same assay was used with selective inhibition of lipoprotein lipase by protamine sulfate (22). Subtraction of h-TGL from PHLA gives information about the LPL activity.

Apolipoprotein A1 and A2 were measured using a modification of the immunoturbidimetric assay, which was based on an immunonephelometric technique (23). Apolipoprotein B was measured by radial immunodiffusion.

The fatty acid composition of the plasma cholesteryl esters was analyzed by gas-liquid chromatography (24). Erythrocyte membrane lipid analyses were performed as described previously (25). The fatty acid composition of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) were analyzed separately. The phospholipid unsaturation was calculated from the total fatty acid composition of PC and PE and was expressed as double bond index (DBI: mean of double bonds per molecule of fatty acid).

Statistical analysis

This study was designed as a crossover clinical trial with two study periods of 30 wk each. From the multiple measurements of the variables during the two study periods, average values of the three final measurements obtained between weeks 18 and 30 were calculated. For the analysis, the data from the two sequence groups were pooled. For the statistical evaluation of the treatment responses analysis of variance according to Hills and Armitage was performed (26). This evaluation included the

DIETARY LINOLEIC ACID IN NIDDM

451

TABLE 3

Fatty acid composition of cholesteryl esters during the study periods with a low P:S and high P:S diet (mean values of three assessments at weeks 18, 24, and 30)*

Fatty acid		Low P:S diet	High P:S diet
		mol %	mol %
Palmitic	(C _{16:0})	13.0 ± 0.4	12.4 ± 0.2
Palmitoleic	(C _{16:1})	3.9 ± 0.5	3.3 ± 0.5†
Stearic	(C _{18:0})	1.1 ± 0.1	1.1 ± 0.1
Oleic	(C _{18:1})	18.4 ± 0.6	15.7 ± 0.9‡
Linoleic	(C _{18:2})	55.8 ± 1.5	59.8 ± 2.0‡
Linolenic	(C _{18:3})	1.1 ± 0.1	1.0 ± 0.2
Homogammalinolenic	(C _{20:3})	0.6 ± 0.1	0.6 ± 0.1
Arachidonic	(C _{20:4})	5.1 ± 0.3	5.0 ± 0.4
Eicosapentaenoic	(C _{20:5})	0.7 ± 0.1	0.7 ± 0.1
Docosahexaenoic	(C _{22:6})	0.3 ± 0.1	0.4 ± 0.1
DBI§		1.65 ± 0.02	1.70 ± 0.01†

* ± 1 SEM.

† p < 0.01.

‡ p < 0.001 as compared to mean value during low P:S diet.

§ DBI (Double Bond Index) = mean of double bonds per molecule fatty acid.

analysis of the sequence effect on the results. All results are presented as means ± SEM.

Results

Diet compliance was determined with two independent methods: 1-wk dietary recalls at weeks 6, 12, and 24 and assessment of the fatty acid composition of the cholesteryl esters at 6-wk intervals (Tables 2 and 3). The cholesterol intake was lower during the high P:S as compared with the low P:S diet ($p < 0.05$). The composition of the diet remained otherwise unchanged (Table 2). These dietary recall data were confirmed by the fatty acid composition of the cholesteryl esters, which demonstrated a significant increase of the linoleic acid (C_{18:2}) content, mainly at the expense of palmitoleic acid (C_{16:1}) and oleic acid (C_{18:1}). These changes resulted in a significant increase of the DBI (Table 3).

The relevant fatty acid content in erythrocyte PC and PE are given in Table 4. The high P:S diet caused a significantly higher stearic acid (C_{18:0}) and linoleic acid (C_{18:2}) content in PC at the expense of C_{16:0} and oleic acid. In erythrocyte PE the high P:S diet, as compared with the low P:S diet, resulted in a significantly higher linoleic acid and stearic acid content and a lower oleic acid content. In neither PC nor PE was a change seen in the total ω-3 fatty acid content or in the DBI.

Mean body weights were not significantly different for either dietary period: 77.9 ± 4.0 vs 78.0 ± 4.1 kg for the low vs high P:S diets, respectively. Mean systolic and diastolic blood pressures were not affected by the change in dietary linoleic acid intake: 137.6 ± 4.4 vs 138.1 ± 3.8 mm Hg and 84.8 ± 2.0 vs 85.0 ± 2.0 mm Hg for the low and high P:S diets, respectively.

Serum lipoprotein, apolipoprotein, and triacylglycerol levels are given in Figure 1 and Table 5. Total cholesterol and LDL-cholesterol levels were significantly higher during the low P:S as compared with the high P:S diet (Table 5). The differences in total cholesterol level were only obvious from 12 wk onward (Fig 1). Apolipoprotein B levels tended to be lower during the high P:S diet than during the low P:S diet but the mean levels during the final 12 wk were not significantly different for either dietary period. Serum VLDL, HDL2 and HDL3 cholesterol, apolipoprotein A1 and A2, and triacylglycerol levels were not significantly different for the low and high P:S diets.

The PHLA and the activities of hepatic and LPL were not influenced by the change in P:S of the diet: 19.9 ± 1.2 vs 20.0 ± 0.6, 6.4 ± 0.7 vs 6.7 ± 0.6, and 13.5 ± 0.8 vs 13.3 ± 0.9 mmol · L⁻¹ · h⁻¹ for the low and high P:S diets, respectively.

Fasting blood glucose levels and glycosylated hemoglobin percentages were not significantly different for the low and high P:S diets: 10.8 ± 0.7 vs 10.5 ± 0.7 mmol/L and 9.2 ± 0.7 vs 9.2 ± 0.7%, respectively.

Incremental blood glucose, plasma C peptide, and insulin responses to the standardized liquid mixed meal during the final week of the study periods were not significantly different for either diet: 577 ± 91 vs 597 ± 79 mmol · L⁻¹ · min⁻¹, 5199 ± 1260 vs 4531 ± 909 mU · L⁻¹ · min⁻¹, and 169 ± 31 vs 175 ± 33 nmol · L⁻¹ · min⁻¹ for the low and high P:S diets, respectively.

In vitro binding of ¹²⁵I-labeled insulin at tracer concentrations to red blood cells was significantly greater during the dietary period with a high linoleic acid intake as compared with the saturated fat intake: 7.4 ± 1.3 vs 6.2 ± 0.2% ($p = 0.003$).

In vivo insulin sensitivity was assessed by constructing insulin dose-response curves at three different infusion rates. The fasting plasma insulin levels at the end of each dietary period were identical during the low and high P:S diet: 9 ± 1 vs 9 ± 1 mU/L. The plasma insulin levels achieved during the dietary periods with a low and high P:S ratio were not significantly different for the three insulin infusion rates (Table 6), permitting comparison of the metabolic clearance rate of glucose. From the blood glucose levels achieved during the final 30 min of each glucose-insulin infusion, the metabolic clearance rate (MCR) of glucose was calculated. The MCR of glucose was significantly higher at the insulin infusion rate of 50 mU · kg⁻¹ · h⁻¹ during the dietary period with a high P:S as compared with a low P:S diet (Table 6). At the two higher insulin infusion rates the MCRs of glucose were not significantly different for either dietary period.

The serum lipids and insulin sensitivity variables responded not significantly different in subgroups of patients that were formed according to age, body mass index (BMI), sex, and fasting blood glucose level.

Discussion

The increased risk of ischemic heart disease in diabetics has been attributed to the diabetic state per se, includ-

TABLE 4
Relevant fatty acid content in erythrocyte phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the final week of the study periods with a low and high P:S diet*

Fatty acid	PC		PE	
	Low P:S diet	High P:S diet	Low P:S diet	High P:S diet
mol %				
Stearic (C _{16:0})	38.7 ± 0.4	37.9 ± 0.6†	18.7 ± 0.4	18.3 ± 0.4
Oleic (C _{18:1})	10.8 ± 0.3	11.6 ± 0.4‡	7.6 ± 0.2	8.0 ± 0.2†
Linoleic (C _{18:2})	15.5 ± 0.6	14.1 ± 0.8‡	16.6 ± 0.4	15.7 ± 0.4‡
Arachidonic (C _{20:4})	23.4 ± 1.0	24.7 ± 1.4†	7.0 ± 0.4	7.7 ± 0.6†
ω-3§	4.8 ± 0.3	4.8 ± 0.3	18.8 ± 0.4	18.7 ± 0.4
DBI	2.2 ± 0.1	2.4 ± 0.2	9.9 ± 0.6	9.3 ± 0.7
	1.01 ± 0.01	1.04 ± 0.01	1.87 ± 0.02	1.86 ± 0.02

* $\bar{x} \pm 1$ SEM.

† $p < 0.05$.

‡ $p < 0.01$.

§ ω-3 = omega 3 fatty acids, ie, sum of 20:5, 22:5, and 22:6 fatty acids.

|| DBI (Double Bond Index) = mean of double bonds per molecule fatty acid.

ing hyperglycemia and hyperinsulinemia and to abnormalities in lipid metabolism (1-3, 9, 10, 27-29). The relationship between the development of atherosclerotic complications and the existence of hyperlipidemia is complex although the latter is suspected to contribute to the high prevalence of macrovascular complications in diabetes (6, 7, 9, 30, 31).

From the dietary record data and the plasma cholesterin ester analyses and the stable weight throughout the study periods it was concluded that the compliance of the patients to the prescribed diets was excellent, which probably was due to the simplicity of the dietary alteration, ie, substitution of linoleic-acid-enriched oils and margarines for saturated fatty acids. The major advantage of this study design is that the observed treatment effects can be attributed to changes in the linoleic-acid content of the diet only.

The cholesterol intake during the dietary period with a low P:S ratio was higher than during the use of the linoleic-acid-enriched diet, probably because of the use of butter rather than margarines and vegetable oils. However, it is very unlikely that a difference in cholesterol consumption of 50 mg/d will affect the lipoprotein levels (32, 33).

The differences in P:S of the diet were also reflected by changes in proportional fatty acid contents of PC and PE in the erythrocyte membrane. Linoleic-acid enrichment of the diet resulted in an increase of linoleic acid in both PC and PE phospholipid classes, mostly at the expense of oleic acid. The lower intake of saturated fatty acids during the high P:S diet resulted in a lower C_{16:0} content, which seemed to be compensated for by a proportional increase of stearic acid. The sums of the C_{16:0} and stearic and of the oleic and linoleic fatty acid contents in PC and PE remained remarkably similar for both dietary periods, supporting the hypothesis that cells regulate to some

extent their membrane lipid composition in an attempt to control membrane fluidity (34).

Hypertension is a known risk factor for cardiovascular disease in nondiabetic and diabetic patients (1, 2, 9). Risk of cardiovascular disease increases with increasing blood pressure. Therefore, it seems that already small reductions in blood pressure can be favorable. Another study (35) demonstrated that a diet low in fat (~23% of energy intake) with a P:S ratio of ~1 and enriched with vegetables has a modest lowering effect on the blood pressure during a 6 wk follow-up study as compared with a conventional diet (dietary fat 40 energy %, P:S 0.27) in subjects. It was suggested that the increase in the P:S was the major dietary change responsible for the lowering of the blood pressure. In our long-term study no change in blood pressure was observed during the dietary period with a high P:S, suggesting that linoleic-acid enrichment of the diet has no blood pressure lowering effect. Therefore, it seems more likely that other components that were altered in the study by Puska et al (35), eg, dietary fibers (36) and total fat content (37), must be held responsible for the observed effect.

In our study no changes were observed in glycemic control, as reflected by fasting blood glucose levels and proportions of glycosylated hemoglobin, during either dietary period. Previous studies have suggested that substitution of sunflower oil for saturated fatty acids causes a decrease of insulin requirements in diabetic patients (13, 38). Houtsmuller (38) found modest improvements in blood glucose profiles in seven obese noninsulin-dependent diabetics in metabolic ward conditions during 10 d of feeding experiments comparing 50 energy % sunflower oil with 50 energy % butter. These short-term experiments comparing extreme P:S are obviously not comparable with our long-term study in which acceptable and practically maintainable diets in normal daily

DIETARY LINOLEIC ACID IN NIDDM

453

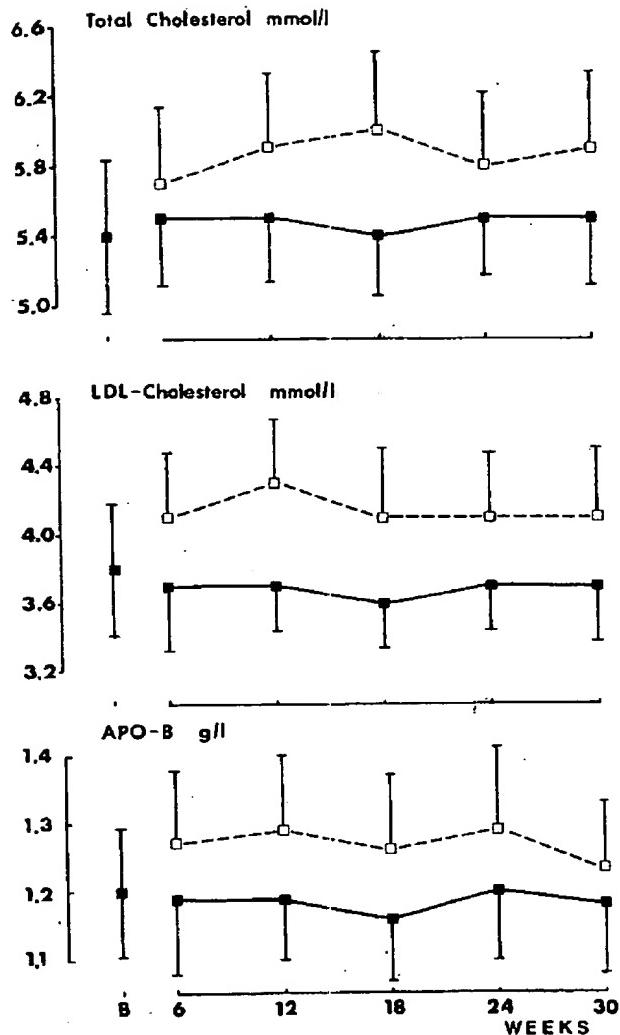


FIG 1. Total and LDL-cholesterol and apolipoprotein B (apo-B) serum levels at base line (B) and during the use of the low (□- - -□) and high P:S (■—■) diets for 30 wk ($\bar{x} \pm$ SEM).

life situations were investigated. From our study we may conclude that linoleic-acid enrichment to an amount of ~10 energy % without altering the other components of the diet has no effect on glycemic control in moderately well controlled noninsulin-dependent diabetic patients. Our data are in agreement with a recent study comparing a low carbohydrate (LC) diet (40 energy % fat with a P:S of 0.3) with a modified fat (MF) diet (30 energy % fat with P:S of 0.9) in a group of 149 noninsulin-dependent diabetic patients. During a 5-y follow-up no differences in glycemic control could be demonstrated between the LC and MF dietary groups (39).

One of the major pathophysiologic factors responsible for the diabetic state in noninsulin-dependent diabetes is insulin resistance (40). The impaired insulin action has

been attributed to defects at the binding site (insulin receptor) and to impeded intracellular glucose metabolism (postbinding defect). In this study we assessed the specific binding of ^{125}I -labeled insulin to red blood cells, as a measure of insulin receptor binding capacity (15). During the high P:S diet a significantly higher insulin binding was found as compared with the low P:S diet, which may be explained by alterations in the phospholipid milieu in which the insulin receptor is embedded. This finding is in agreement with the results of Ginsberg et al (41) who demonstrated an increase of the total number of insulin receptors on Friend erythroleukemic cells in media causing an enhancement of unsaturated fatty acids in cell membranes. However the insulin binding data do not necessarily give information on the *in vivo* insulin sensitivity. The fact that no direct correlation has been demonstrated between insulin-binding properties of blood cells and adipocytes in noninsulin-dependent diabetes has casted considerable doubt on its use as a measure of the insulin binding characteristics of insulin-sensitive tissues, which are relevant to carbohydrate metabolism *in vivo* (42).

In vivo insulin sensitivity was measured by constructing insulin dose-response curves. Insulin action was expressed as the MRC of glucose during the glucose-insulin infusions at prefixed rates. This method has been demonstrated to give comparable results with the glucose clamp technique in subjects with normal and abnormal glucose tolerance. In a previous study (16) we found in normal volunteers an increase of the mean MCR of glucose from 10.8 ± 0.6 to $20.0 \pm 1.2 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ when enhancing the insulin infusion rate from 50 to 500 $\text{mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ whereas the mean insulin dose-response curve of the patient in the present study was similar to the mean curve of a group of noninsulin-dependent diabetics which reflected impeded insulin action. The high P:S diet caused a small but significant improvement of the insulin action at insulin levels of ~50 mU/L without affecting the MCR of glucose at the higher insulin levels.

TABLE 5
Serum concentrations of lipoproteins and apolipoproteins during the study periods with a low and high P:S diet (mean values of three assessments at weeks 18, 24, and 30)*

	Low P:S diet	High P:S diet	Percent
Total cholesterol (mmol/L)	5.92 ± 0.45	$5.47 \pm 0.35\ddagger$	7.6
VLDL cholesterol (mmol/L)	0.74 ± 0.15	0.68 ± 0.14	
LDL cholesterol (mmol/L)	4.08 ± 0.38	$3.68 \pm 0.28\ddagger$	9.8
HDL2 cholesterol (mmol/L)	0.37 ± 0.04	0.38 ± 0.05	
HDL3 cholesterol (mmol/L)	0.73 ± 0.04	0.74 ± 0.04	
Triacylglycerol (mmol/L)	2.42 ± 0.41	2.22 ± 0.34	
Apolipoprotein A1 (g/L)	1.06 ± 0.04	1.10 ± 0.03	
Apolipoprotein A2 (g/L)	0.35 ± 0.02	0.36 ± 0.02	
Apolipoprotein B (g/L)	1.26 ± 0.11	1.18 ± 0.09	

* $\bar{x} \pm 1$ SEM.

† $p < 0.01$ as compared with mean value during low P:S diet.

TABLE 6

Plasma insulin levels and metabolic clearance rates (MCR) of glucose during the sequential glucose-insulin infusions in the final week of the study periods with a low and high P:S diet*

Glucose $mg \cdot kg^{-1} \cdot min^{-1}$	Insulin $mU \cdot kg^{-1} \cdot h^{-1}$	Plasma insulin		MCR of glucose	
		Low P:S diet mU/L	High P:S diet mU/L	Low P:S diet $ml \cdot kg^{-1} \cdot min^{-1}$	High P:S diet $ml \cdot kg \cdot min^{-1}$
6	50	50 ± 6	50 ± 4	3.7 ± 0.6	4.9 ± 0.9†
8	150	168 ± 19	161 ± 16	9.7 ± 1.4	10.2 ± 1.4
10	500	1004 ± 114	971 ± 95	15.4 ± 2.3	15.5 ± 1.8

* $\bar{x} \pm 1 SEM$.

† $p < 0.05$ as compared with mean value during low P:S diet.

It seems from the in vitro and in vivo data that linoleic acid enrichment of the cell membranes may affect the insulin receptor binding capacity, possibly by influencing the fatty acid composition of the cell membranes (41). However the major impedance in insulin action was not alleviated and therefore it was not to be expected that the small enhancement of insulin action at the low insulin levels was translated into an improved glycemic control.

The meal tolerance test demonstrated very similar incremental glucose, insulin, and C peptide responses to a liquid mixed meal during the final week of each dietary period, confirming the lack of a major change in insulin sensitivity and demonstrating an unaltered β cell function and hepatic insulin extraction. Therefore, it seems very unlikely that previously reported improvement of glucose tolerance in noninsulin-dependent diabetics by use of a modified fat diet is due to the linoleic acid enrichment only (14).

The serum total and LDL-cholesterol levels were significantly lower during the high P:S as compared with the low P:S diet. This difference seems to be due mainly to a rise of the serum total and LDL-cholesterol levels during the low P:S diet (Fig 1). Other studies in nondiabetic subjects, which compared diets with similar P:S and fat contents as in our study, demonstrated greater differences in total and LDL-cholesterol levels than those we found (43, 44).

Furthermore, a significant decline of apolipoprotein B levels is usually observed in nondiabetic subjects who eat diets with a high P:S ratio (43–46). Therefore, it seems that noninsulin-dependent diabetics respond differently and possibly less effectively to changes in linoleic acid content of the diet than nondiabetic subjects. Our patient population demonstrated a wide range in age, BMI, and fasting blood glucose levels. However, their serum lipid levels responded to the change in the P:S of the diet in a fairly uniform way. Also, the female and male patients did not respond significantly different to the dietary alteration. The Lipid Research Clinical Trials (47) have demonstrated that each percent reduction of the LDL-cholesterol level was associated with a 2% decrease of the incidence of coronary heart disease. Because it is

suspected that also in diabetes disordered lipoprotein metabolism may contribute to the high prevalence of macrovascular complications, it is of great interest that linoleic-acid enrichment of the diet resulted in a mean decrease of LDL cholesterol levels by 9.8% without affecting HDL2-cholesterol levels. The catabolism of the LDL particle is mediated mainly by LDL-receptor mediated pathways (48). Because one of the explanations for the cholesterol lowering effect of a high P:S diet is an enhanced LDL clearance, it is tentative to speculate that an increase of the linoleic acid content of the cell membranes may stimulate, as suggested for the insulin binding site, the activity of the LDL receptors (49). Serum levels of other major lipoproteins, triacylglycerol, and apolipoproteins A1 and A2 were not affected by the change in P:S of the diet. This finding is in agreement with previous observations (12, 44, 46) in nondiabetic subjects. Other investigators have found a triacylglycerol lowering effect and decreased HDL-cholesterol levels in healthy and hyperlipidemic subjects after high P:S diets (50, 51). These discrepancies in results may be explained by differences in composition and the P:S of the diets and by the heterogeneity of lipid disorders in the study populations. Also, the duration of the investigations might be a very important determinant for the outcome of study results. The diet-induced change in total cholesterol levels was only obvious 12 wk after initiating the change in the P:S. This raises some doubt on the interpretability of shorter term dietary studies.

The PHLA was not affected by the linoleic-acid enrichment of the diet nor were the LPL and hepatic lipase activity different for either study period. Low HDL2 concentrations in diabetes may be caused by subnormal LPL and/or high hepatic lipase activity. HDL is derived from excess surface constituents, which are released after LPL-induced hydrolysis of triacylglycerol rich particles and its further metabolism is mediated by hepatic lipase (8). LPL activity is regulated by insulin. Insulin treatment in noninsulin-dependent diabetics causes enhanced LPL activity, reflected by an increase in HDL cholesterol and a decline in triacylglycerol levels (52). The absence of an effect of linoleic acid on LPL and hepatic lipase activity

is in a
yglyc
Ta
resul
out a
Glyc
The
side
ther
ever

Ti
ackn
their
insu
mea
stati

Re:
1.
2.
3.
4.
5.
6.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.